REMARKS

Reconsideration of the claims of the instant application is respectfully requested in view of the above amendments and the following remarks. Claims 1 and 4 are amended. Claims 21-30 are added. The support for the amendments to claim 1 is found at least on pages 6 and 11 of the substitute specification submitted herewith. The support for the new claims is found at least on pages 6, 11, and 45 of the substitute specification. Accordingly, the amendment to claim 1 and addition of claims 21-30 do not introduce new matter.

The claims of the instant invention are drawn, in one aspect, to a method of detecting hepcidin comprising obtaining a tissue or fluid sample from a subject; and contacting the sample with an antibody or fragment thereof that specifically binds to one or more carboxy terminal epitopes of SEQ ID NO: 2; wherein the tissue or fluid sample is selected from a kidney sample, a liver sample, a urine sample, a serum sample, and a blood sample.

Amendments to the drawings.

During the phone conversation, the Examiner noted that figures 6-9 are missing. Applicants thank the Examiner for noticing this omission and submit replacement drawings. In these replacement drawings, figures 1 through 5 are unchanged. Figure 6 corresponds to Fig. 6 of U.S. Application 10/299,486 which is a parent of the instant application. Original Figures 7 and 8 are not submitted. Newly submitted Fig. 7 corresponds to the description of Fig. 9 and derives support from Figure 7 of U.S. Application 10/299,486 which is a parent of the instant application. Figures 10-16 have been renumbered as Figures 8-14, respectively. Accordingly, no new matter has been added by the amendment to the figures.

Amendments to the specification.

The specification has been replaced. The substitute specification reflects the changes in figure numbering (e.g., the removal of description of old Figures 7 and 8 from the section "Brief Description of the Drawings") and ensures compliance with sequence listing requirements. Thus, the substitute specification does not introduce any new matter.

.

Double Patenting Rejections

The Examiner provisionally rejected the claims of the instant application on the ground of non-statutory obviousness-type double patenting over claims 1, 3, and 4 of U.S. Application 10/299,486.

Applicants thank Examiner Counts for calling the Applicants' attorney on March 13, 2008 in connection with prosecution of application 10/299,486. During that phone conversation, it was agreed that claims 1, 3, and 4 of application 10/299,486 would be cancelled by the Examiner's amendment. Accordingly, Applicants respectfully request the Examiner to withdraw this ground for rejection.

Rejection based on 35 U.S.C. § 112, paragraph 1 (Written Description)

The Examiner rejected claim 4 as allegedly failing to comply with the written description requirement. Without agreeing with the Examiner, and solely in the interest of expediting the prosecution of the instant application, claim 4 has been amended. Applicants submit that the amendment overcomes this ground for rejection and respectfully request the Examiner to withdraw the rejection of claim 4 based on 35 U.S.C. § 112 (written description).

Rejection based on 35 U.S.C. § 112, paragraph 1 (Enablement)

The Examiner argues that the claims of the instant application are not enabled. In support of this assertion, the Examiner advances several arguments.

First, the Examiner asserts that the specification does not enable a person of ordinary skill in the art to use any and all samples for hepcidin detection. Applicants thank the Examiner for agreeing that the disclosure of the instant application enables detection of hepcidin in liver, kidney, and urine samples. As discussed above, claim 1 has been amended to recite these three sources of hepcidin.

In addition to urine, kidney, and liver samples, claim 1 also recites samples of blood. Applicants respectfully disagree with the Examiner's conclusion that the detection of hepcidin in blood samples by antibodies against the carboxy-terminus of SEQ ID NO: 2 is not enabled. In support of their position, Applicants respectfully bring the

Examiner's attention to paragraph 115 of the Application as filed (US 20070092916), the relevant part of which is recited as follows:

> [a]lthough the C-terminal antibody EG(1)-HepC revealed specific results in dot blot, Western blot, immunohistochemistry and immunofluorescence experiments (FIGS. 1-5), it did not work in ELISA. The compact folding pattern of hepcidin and its tertiary structure in the blood may account for the inability of the EG(1)-HepC antibody to identify circulating hepcidin.

This paragraph is recited under a subheading "Detection of Hepcidin Propeptide in Human Plasma." Accordingly, the statement quoted above is devoted to the detection of hepcidin in blood. The recited quote explicitly states that the antibody to the carboxy-terminus of SEQ ID NO: 2 detects hepcidin in "in dot blot, Western blot, immunohistochemistry and immunofluorescence experiments." Accordingly, Applicants respectfully submit that the instant disclosure enables one of skill in the art to detect hepcidin in blood using antibodies to carboxy-terminus of hepcidin disclosed in the instant application.

Second, the Examiner argues that the specification does not provide enablement for diagnosing any and all disease conditions.

With regard to the scope of the claims, the meaning of term "indicative" as used in claim 1 is consistent with Steadman's Medical Dictionary, 22nd Ed. (see the definition of "indication" on p. 629, attached hereto as Exhibit A) which defines "indication" as "suggestion or pointer." Accordingly, the detection of the abnormal hepcidin level does not need to conclusively prove that the patient has certain disease or condition, but rather may serve as an additional criterion for diagnostics of that disease. It is a normal diagnostic practice that the results of several different tests are taken together to conclusively prove whether a patient has the disease in question. Accordingly, if it is known that certain disease or condition is characterized by abnormal hepcidin levels, a person of ordinary skill in the art (and in this case, the ordinary skill in the art is, unquestionably, high) would consider the abnormal hepcidin level in diagnosing a disease, such as those recited in claim 1.

Second, Applicants respectfully bring to the Examiner's attention MPEP § 2164.03 which states, in relevant part, as follows:

> The amount of guidance or direction needed to enable the invention is inversely related to the amount of knowledge in the state of the art as well as the predictability in the art. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The "amount of guidance or direction" refers to that information in the application, as originally filed, that teaches exactly how to make or use the invention.

Applicants respectfully submit that the determination of compliance with the enablement requirement should be done not in vacuum but considering the knowledge accumulated in the prior art as well as the level of ordinary skill in the art. In this case, the level of ordinary skill is undoubtedly high.

In addition, as discussed in details below, at the time of filing, it was well established fact that hepcidin is a major regulator of iron homeostasis, and that the diseases recited in the claims are characterized by and/or result from abnormal iron levels. Thus, Applicants submit that in view of the predictability in the art and high level of ordinary skill, extensive guidance and direction are not needed.

Applicants also thank the Examiner for agreeing that the application enables diagnosis of hereditary hemochromatosis, chronic renal insufficiency, and renal anemia. Applicants note, however, that narrow interpretation of the enablement requirement is contrary to the spirit of 35 U.S.C. § 112, first paragraph, as evidenced by MPEP § 2164.08 (*Internal quotations omitted*):

> [T]o provide effective incentives, claims must adequately protect inventors. To demand that the first to disclose shall limit his claims to what he has found will work or to materials which meet the guidelines specified for "preferred" materials in a process such as the one herein involved would not serve the constitutional purpose of promoting progress in the useful arts.

In the instant case, Applicants respectfully bring to the Examiner's attention multiple articles establishing connection between abnormal hepcidin levels and at least some of the diseases recited in the claims, either directly or through the knowledge in the art that these diseases are characterized by abnormal iron metabolism and that hepcidin is a major regulator of iron homeostasis, and is regulated by iron. In addition, hepcidin is morphofunctionally coupled to TfR2, which is regulated by transferrin saturation and, in turn, modulates expression of hepcidin. Applicants respectfully note that the evidence of the correlation between abnormal hepcidin level and/or abnormal iron level and the

recited diseases is not limited to the articles recited in Table 1 below; rather, the recited articles serve as non-limiting examples. All references, except Sanchez and Thiele, which have not been previously submitted to the Patent Office are submitted with the Information Disclosure Statement accompanying this response.

Table 1: Association between diseases and hepcidin level and/or iron level.

Disease or Condition	**Reference**
Iron deficiency or overload	Nicolas et al; *Proc Natl Acad Sci U S A*. 2001 Jul 17;98(15):8780-5. Epub 2001 Jul 10; ("a complete defect in hepcidin expression is responsible for progressive tissue iron overload.") Pigeon et al, *J Biol Chem.* 2001 Mar 16;276(11):7811-9. Epub 2000 Dec 11 ("diseases associated with iron overload are common in humans and can be responsible for the shortening of life expectancy. Genetic hemochromatosis is characterized by digestive hyperabsorption of iron. In 1996, Feder *et al.* reported that the C282Y mutation of *HFE* gene was responsible for this disease. Other iron overload diseases include β-thalassemias, genetic diseases characterized by hemolytic anemia, in which iron overload results both from digestive hyperabsorption of iron and blood transfusions. In addition, it has been shown that iron excess may occur in chronic liver diseases such as alcoholic liver diseases or hepatitis B and C infections.")
Genetic and nongenetic iron overload diseases	See Pigeon et al, supra See Nicolas et al (2001) supra Ravel, Clinical Laboratory Medicine: Clinical Application of Laboratory Data, 6th Ed. Mosby- Year Book, Inc. 1995, p. 605 ("Serum iron levels can also be increased in chronic hepatitis B or C infection (46% of cases in one study) and in hemosiderosis (nonhereditary iron overload) due to blood transfusions, chronic severe hemolytic anemias, sideroblastic anemias, alcoholic cirrhosis, parenteral iron therapy, and considerably increased iron intake.")
Iron deficiency anemia	Nicolas et al; *Proc Natl Acad Sci U S A*. 2002 Apr 2;99(7):4596-601 ("The classical hematological features of iron deficiency anemia associated with the severe iron deficit in transgenic mice strongly support the idea of hepcidin acting as a negative regulator of iron transport').
Inflammations or infections	Park et al, *J. Biol. Chem* 276: 7806-1810 2001 ("we characterized a cysteine-rich peptide with three forms differing by amino-terminal truncation, and we named it hepcidin (Hepc) because of its origin in the liver and its antimicrobial properties"); Nicolas et al (2002) supra ("Indeed, the hepcidin gene was reported to be responsive to different stimuli; in particular, it was associated with inflammation.")

Microcytic Anemia	Nicolas et al (2002) supra ("Severe Microcytic Anemia in Transgenic Mice Overexpressing Hepcidin")
Hepcidin is regulated by iron	Pigeon et al, *supra* ("liver iron excess itself is responsible for the up-regulation of mRNA expression") Nicolas et al (2001), supra ("a complete defect in hepcidin expression is responsible for progressive tissue iron overload")
Hereditary hemochromatosis	See Nicolas et al (2001) supra ("The striking similarity of the alterations in iron metabolism between *HFE* knockout mice, a murine model of hereditary hemochromatosis, and the *Usf2*$^{-/-}$ hepcidin-deficient mice suggests that hepcidin may function in the same regulatory pathway as HFE")
Other Hematological disases and/or Erythropoiesis abnormalities	See Nicolas et al (2002), supra ("If hepcidin compromises iron transport, then erythropoiesis should be altered because most iron is directed to hemoglobin synthesis.") Weinstein et al., Blood, 15 November 2002, Vol. 100, No. 10, pp. 3776-3781; pre-published on Jun. 28, 2002 (The anemia of chronic disease "is characterized by a blunted erythropoietin response by erythroid precursors, decreased red blood cell survival, and a defect in iron absorption and macrophage iron retention, which interrupts iron delivery to erythroid precursor cells").
Liver diseases	See Pigeon et al, supra.
Anemia of chronic disease	See Weinstein et al., supra. ("hepcidin plays a major, causative role in the anemia observed in our subgroup of patients with hepatic adenomas, and we speculate that it is important in the pathogenesis of the anemia of chronic disease in general.")
Tumors	See Weinstein et al, supra ("Studies of her [the patient's] liver showed inappropriately high expression of hepcidin mRNA in adenoma tissue.")
Immunological disorders	See Weinstein et al., supra ("The anemia of chronic disease is a prevalent, poorly understood condition that afflicts patients with a wide variety of diseases, including infections, malignancies, and *rheumatologic disorders*.") Italics added.
Hemosiderosis	See Ravel, supra.
Secondary hemochromatosis	Beutler et al, Drug Metab Dispos. 2001 Apr; 29(4):495-9, table II.
Aceruloplasminemia	Beutler et al, supra ("Iron overload occurs in a number of hereditary disorders including atransferrinemia, aceruloplasminemia, X-linked hereditary sideroblastic anemia, thalassemia major, congenital dyserythropoietic anemia, and various red cell enzyme deficiencies.")
Hypotransferrinemia	Malecki EA, Devenyi AG, Beard JL, Connor JR. Biometals. 1998 Sep;11(3):265-76. "Transferrin (Tf) is the major iron transport protein in plasma… Plasma concentratiopns of Tf and synthesis in liver may be upregulated during dietary iron deficiency, but the

	mechanism of this regulation is not well inderstood." (internal quotations omitted) Also "Plasma Tf concentrations were lower in +/hpx [hypotransferinemic] mice, plasma Tf nearly doubled and liver Tf was only 50% of normal in response to iron deficiency." Also "Previous work with mice homozygous for the mutation has shown that they are severely anemic, yet accumulate iron in the liver." (internal quotation omitted) Craven et al., *Proc Natl Acad Sci U S A*. 1987 May;84(10):3457-61 ("Congenital hypotransferrinemia is a rare human genetic disorder characterized by a severe deficiency in serum transferrin. The defect causes iron deficient erythropoiesis and marked iron deficiency anemia and severe iron overload in all non-hematopoietic tissues. A similar phenotype has been observed in the hypotransferrinemic (hpx/hpx) mice that provide a model to help understand the human disease and iron homeostasis. The presence of severe anemia in the hpx/hpx mice and patients with congenital hypotransferrinemia indicates that very little iron enters erythroid precusors through non-transferrin cycle pathways.") Internal quotations omitted
Atransferrinemia	See Beutler et al; supra
Sideroblastic anemia	See Beutler et al; supra
Thalassemia	See Beutler et al; supra
Anemia with reticulocytosis	Hartman KR, Barker JA. Am J Hematol. 1996 Apr;51(4):269-75. Murphy PT, Hutchinson RM. Drugs Aging. 1994 Feb;4(2):113-27. Review (See p. 274, Right Col. Second full paragraph discussing a mouse model of microcytic anemia and disclosing that in these mice "uptake [of iron] by reticulocytes is impaired.")
Leukemia	See Ravel, supra, at page 67 ("Anemia is present in about 90% of patients [with acute leukaemia] and is generally of moderate or severe degree.")
Polyglobulie	Sánchez Sánchez ML et al., An Med Interna. 1993 Aug;10(8):377-80. Spanish. Thiele J, Kvasnicka HM. Pathologe. 2000 Jan;21(1):24-30. Review. German.
Macrocytic anemia	Irwin JJ, Kirchner JT. *Am Fam Physician* 2001;64:1379-86 ("The use of the mean corpuscular volume to classify the anemia as microcytic, normocytic or macrocytic is a standard diagnostic approach." Also "Other possible causes include chronic liver disease, hypothyroidism and myelodysplastic disorders.") Fargion S al., *Blood*. 2000 Nov 15;96(10):3653-5. ("Herein is described a case of a young woman presenting with iron overload and macrocytosis" See also figure 1A and description thereof. Also "A complete reevaluation confirmed the presence of macrocytosis (MCV 104), mild anemia (Hb 11.6), and iron

	overload…")
Hemolytic anemia	See Pigeon et al; supra.
Normocytic anemia	Irwin JJ, Kirchner JT, supra ("The use of the mean corpuscular volume to classify the anemia as microcytic, normocytic or macrocytic is a standard diagnostic approach." Goodnough LT, Skikne B, Brugnara C. *Blood*. 2000 Aug 1;96(3):823-33. Review. " A distinguishing characteristic of the anemia in the patients undergoing chronic renal dialysis is the presence of a normal mean corpuscular volume (MCV) in 85% of the patients." Also "Anemic patients undergoing dialysis may have suboptimal responses to oral iron therapy for several reasons.") Brill JR, Baumgardner DJ. *Am Fam Physician*. 2000 Nov 15;62(10):2255-64. Review. (See, e.g., Table 1 disclosing that anemia of chronic disease, renal failure, liver disease, and sideroblastic anemias are among the causes for normocystic anemia. Also "Anemia of chronic disease, the most common normocystic anemia is found in 6 percent of adult patients hospitalized by family physicians.")

Accordingly, the relevant art (diseases associated with abnormal hepcidin level or diseases associated with iron metabolism disturbances) is abundant and predictable. The diseases recited in claims have been shown to be associated either with iron metabolism or with non-physiological level of hepcidin. Thus, based on these articles and knowledge in the art, one of skill in the art would consider the level of hepcidin at least as one of the factors for diagnostics of the diseases recited in claim 1.

Applicants also respectfully note that even if some experimentation may be required, such experimentation is not undue. Assuming, but without admitting, that the experimentation may be complex, it is not necessarily undue. MPEP § 2164.06, specifically mentions that

> In *United States v. Telectronics, Inc.*, 857 F.2d 778, 8 USPQ2d 1217 (Fed. Cir. 1988), *cert. denied*, 490 U.S. 1046 (1989), the court reversed the findings of the district court for lack of clear and convincing proof that undue experimentation was needed. The court ruled that since one embodiment (stainless steel electrodes) and the method to determine dose/response was set forth in the specification, the specification was enabling. The question of time and expense of such studies, approximately $50,000 and 6-12 months standing alone, failed to show undue experimentation.

In this case, Applicants respectfully note that obtaining samples, e.g., urine samples, from patients with the diseases recited in claims, and contacting these samples with the antibodies disclosed in the claims, according to the protocols such as those disclosed in the specification, is no more than routine experimentation.

Applicants further note that the newly added claims 26-30 recite that the tissue or liquid sample is a kidney sample, a liver sample, or a urine sample, which the Examiner has found to be enabled. Further, these claims do not recite disease diagnostics. Accordingly, Applicants respectfully submit that claims 26-30 are enabled.

For at these and other reasons, applicants respectfully submit that the claims of the instant application are fully enabled.

Rejection based on 35 U.S.C. § 112, paragraph 2 (Indefiniteness)

The Examiner rejected the claims of the instant application due to alleged vagueness of the phrase "disease condition." Applicants respectfully disagree that this phrase is vague. Nevertheless, claim 1 has been amended to recite a "method for diagnosing a condition of a disease characterized by non-physiological levels of hepcidin." In this case, the condition is a non-physiological level of hepcidin, and the disease is characterized by this condition.

Applicants respectfully submit that this clarification, especially taken together with the amendment to claim 1, fully addresses the Examiner's concern and respectfully request the Examiner to withdraw this ground for rejection.

Conclusion

In view of these amendments and remarks, applicants believe that this application is in a condition for allowance and an early notice to this effect is earnestly solicited. If the Examiner does not believe that such action can be taken at this time or if the Examiner feels that a telephone interview is necessary or desirable, Applicants welcome the Examiner to call the undersigned at 609-844-3021.

The USPTO is authorized to charge Deposit Account No. 50-1943 for any charges in connection with this matter.

Respectfully submitted,

Date: April 25, 2008

/Vyacheslav Vasilyev/
Vyacheslav Vasilyev
Attorney for the applicant
Registration No. 58,154
Fox Rothschild LLP
Princeton Pike Corporate Center
997 Lenox Drive, Building 3
Lawrenceville, NJ 08648-2311